Glucoamylase Assay

Introduction

In this protocol the glucoamylase activity assay is described. The incubation time and pH is specific optimum for the glucoamylase (glaA) found in *A. niger*. The protocol is adapted from a protocol we received from Kristoffer Bach Falkenberg (PhD student, DTU Biosustain).

Materials

- Plate reader
- 96-microtiter plates for assay
- Starch solution
    - 0.5 g/L starch in 100mM phosphate buffer at optimum pH = 5
- 1M HCl
- Iodine reagent (per 100 mL)
    - 5mM I_2
    - 50mM KI

Procedure:

Prepare reagents and media

1. Prepare all solutions.
2. Autoclave the Starch solution in order to solubilize the starch

Prepare samples

3. Spin down the culture samples.
4. Add the supernatant to the microtiter plate.
    a. There has to be 50 µL sample in each well.
    b. Dilutions are made with phosphate buffer.
5. For the standard curve, add 120 ul of phosphate buffer in wells 2 to 8. In well 1 add 240 µL of starch solution. Transfer from left to right 120 ul and discard the last 120 µL coming from well 7 (don't transfer to well 8). This method results in a linear standard curve with a final volume of 120 µl in each well.

Perform Assay

6. Add 50 µL of the starch solution to each sample well in a 96-microtiter plate (NOT the standard curve wells).
7. Cover the plate with a lid or foil in order to avoid evaporation.
8. Incubate at 40 °C for 10 minutes.
9. Add 20 µL 1M HCl to stop the reaction (NOT the standard curve wells).
10. Add 100 µL iodine reagent and let the colour develop for a few minutes (ALSO the standard curve wells).

11. Measure the absorbance at 580nm.
12. Calculate enzyme activity.
13. U/mL = (A580 control - A580 sample)/((A580/mg starch)*t*V(enzyme)).
    (A580/mg starch is the slope of the standard curve, t is the reaction time and V(enzyme) is the volume of the enzyme added).